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A DEMONSTRATION OF NUMEROUS DISTINCT STRAINS WITHIN THE NOMINAL SPECIES *PESTALOZZIA GUEPINI* DESM.[1]

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In the course of routine pathological work on the plantations of the United States Rubber Company, in Asahan, East Coast of Sumatra, we found that Pestalozzia was among the fungi most often isolated from diseased tissues of *Hevea brasiliensis*. A comparison of several of the strains obtained showed that all were very similar as far as obvious characters were concerned, and all fell within the limits of *Pestalozzia Guepini* Desm., as this species has been broadly interpreted; but they were quantitatively different from one another, and maintained their distinctness through successive generations. Since none of the diseases of rubber which have been attributed to Pestalozzia are particularly important, and since the time at our disposal was limited, only enough measurements were made to demonstrate that the nominal species in this group of fungi may be looked upon as composed of a large number of pure lines, each definable by its mode and range of variation. Looked upon in this light, the Fungi Imperfecti doubtless afford a parallel to the host of constant but hardly perceptibly distinct strains in some groups of the higher plants, such as Hieracium, in which the constancy of the lines is maintained because of the loss of sexual reproduction. The problem of species delimitation in either case is a most difficult one. Among many fungi the close restriction of strains to special hosts gives criteria for the separation of species which have never been differentiated morphologically, although in many such cases morphological as well as physiological distinctions might often be established if more refined biometrical methods were used than those which prevail in systematic work. The truth of this statement, as well as the great advances in mycology which may be expected to follow the more general introduction of biometrical methods, are illustrated by the notable studies of Stakman and Levine (1) in the rusts, and of Rosenbaum (2) in Phytophthora.

Only morphological criteria, however, are available to the systematist in those weakly parasitic genera whose members are not confined to specific, or even closely related, hosts. This fact is coming more and more to be recognized, and mycologists nowadays much less often than formerly attempt to clothe superficial systematic work in the garb of respectability by assuming host delimitation for forms belonging to groups in which no clear case for such delimitation has been made out. Morphologically indistinguishable fungi not known to be confined to particular hosts are best called by the same name. It is idle, for example, to use one name for the Pestalozzia strains causing the gray blight of tea and another for those which cause leaf spot of palms. In both cases, as we have found, the strains show differences among themselves, covering a wide range of variation, just as do the strains from Hevea. Our isolations from hosts other than Para rubber were made in order to find out whether or not the strains infecting these hosts were distinct, and, providing they were, whether or not they had served as sources of infection for Hevea.

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[1] From the Botanical Laboratory of the Hollandsch-Amerikaansche Plantage Maatschappij, Kisaran, Asahan, Sumatra. Published by permission of the United States Rubber Plantations, Inc.

Of all the common hosts of Pestalozzia in the East Indies, it seemed least likely that Hevea would be found to be infected with specifically restricted strains. In fact, it appears that all the common diseases of Para rubber in the Orient are due to more or less ubiquitous fungi, and that no serious, specific diseases of Hevea were introduced from Brazil with the original rubber seed. It is accepted by Burkill (3), who has gone over the existing records with care, that all the oriental Hevea has descended from a single introduction of seeds collected for the Government of British India on the upland plateaus near the valley of the Tapajos River in Brazil. The seeds were grown at Kew, the latter institution having served the purpose of the modern quarantine station, for no diseased stock seemed to have been forwarded to Ceylon and Malaya. In consequence, the causes of rubber diseases in the East are found among those fungi which are little restricted as to host. Generally speaking, the forms pathogenic to Hevea have been reported as causing diseases of the most diverse cultivated plants.

In view of these facts it was natural that we should attempt to identify the Pestalozzia strains of Hevea with those of other plants from which these fungi have been described or reported. As with so many other genera of Fungi Imperfecti, the descriptions of the nominal species of Pestalozzia are generally in no degree diagnostic. There are whole groups of descriptions of which nothing whatever is left, when common factors are canceled, except the names of different host plants and different geographic localities. Quantitative data, when given, are seldom, if ever, stated so that one is convinced of their accuracy.

Pestalozzia is chiefly known in the eastern tropics through causing the gray blight of tea and the leaf spot of the cocoanut palm. The literature shows that several specific names have been applied to the causal fungi with only the vaguest notions of specific delimitations in the group. Of these names the oldest is *Pestalozzia Guepini* Desm. (first published by Desmazières (4) as *Pestalotia Guepini*). It was originally applied to a form or forms from Camellia and Magnolia, characterized by a spore length of about one fiftieth millimeter, with four-septate spores, the terminal and basal cells hyaline, and the three interior cells dark; appendages longer than the spores and typically three in number.

More recently, diseases of several tropical cultivated plants, including the tea blight and a stem disease of rubber seedlings, have been attributed to *Pestalozzia palmarum* Cooke. This name was originally based by Cooke in 1876 (5) upon a fungus from the dead sprout of a cocoanut from Bengal. The spores were stated to be 0.015 mm. long. Later, in 1877, the same author (6) supplemented the description from material found on the dead stems of *Cocos nucifera* from Demerara, giving the length of the colored portion of the spores (*i.e.*, the three inner cells) as 0.045 mm. Cooke was so notoriously careless that it is useless to try to explain the discrepancy in the measurements, or to speculate as to whether or not the material from the two sources was the same. Discarding the measurements as hopelessly irreconcilable, the description affords no distinction from *Pestalozzia Guepini* Desm., except the different host.

In 1872, before Cooke had named *Pestalozzia palmarum* at all, he had described the causal fungus of the gray blight of tea as *Hendersonia theaecola* (7). There is no doubt that this was merely Pestalozzia without the terminal and basal hyaline cells, which fall off in old, weathered specimens. Cooke himself suggested that such was the case, comparing his so-called Hendersonia, in the original description, with *Pestalozzia Guepini*. Recently the tea blight has been christened again by Sawada (8) as *Pestalozzia Theae*. Sawada is hardly to be blamed for neglecting Cooke's defective description and incorrectly formed name, but unfortunately he fails to make out a clear case for the distinctness of the tea fungus. His description (as translated by Tanaka, in Mycologia) gives the length of the spores as 16 to 21 μ, but in a letter dated June 26, 1919, he gives 23 to 31 μ; the latter measurement is doubtless the correct one. Sawada's idea of *Pestalozzia Guepini* is that of a form differing from the tea-blight fungus in having shorter and generally three-septate rather than four-septate spores. Desmazières' description, however, makes it very clear that in the original material of *P. Guepini* the spores were typically four-septate, and therefore five-celled, the three central cells being dark in color. Moreover, his measurement of the length, about one fiftieth millimeter, although vague, appears to be quite as dependable as the measurements of later authors, and would not exclude from *P. Guepini* any of the tropical Pestalozzias which have been isolated from tea, cocoanut, rubber, etc. Sawada states that in *P. Theae* the appendages are club-shaped, whereas in *P. palmarum*, which he records as found upon tea in Formosa, they are filiform. This character is one upon which it seems unwise to place reliance, since any strain may show both conditions, depending upon circumstances. In the last analysis it is the only character used by Sawada for the differentiation of two supposedly distinct fungi found upon the same host in the same locality, and we are unable to grant its validity. The literature shows, as far as tropical diseases caused by Pestalozzia are concerned, that authors have, in general, used the names *P. Guepini* and *P. palmarum* interchangeably, perhaps preferring the former name for tea diseases and the latter for palm diseases, but no one has justified the latter usage either by cross-inoculation studies or by finding dependable morphological distinctions.

We were unable to attempt cross-inoculation studies in the time at our disposal. Likewise, as advocates of biometrical methods in systematic work, we feel called upon to apologize for the scantiness of our statistical data, which were obtained as a by-product, as it were, of other work. We were in general unable to make more than twenty-five measurements of spore length, and an equal number of appendage length, in each generation. However, we have data for each strain covering from four to eight generations. The material proved unusually favorable for statistical study because of the presence of two independent and easily measured variables, namely, spore length and appendage length. In the measurement of spore appendages we chose at random one of the three appendages of each spore, by the simple expedient of measuring whichever appendage lay most perfectly in the focal plane.

In spite of the fact that our procedure might have been improved, we feel that our general conclusion, that the nominal species in the Fungi Imperfecti can be resolved into a large number of quantitatively distinct strains, is well established. Greater refinement of method would merely have increased the significance of the differences between the more closely similar groups of strains, and could only have confirmed the conclusion that might have been drawn from purely theoretical considerations, namely, that in the Fungi Imperfecti, a group of non-sexual organisms, every hereditary modification, of whatever magnitude, must give rise to a pure line. These pure lines are too numerous and too incapable of precise recognition to be named. Consequently the concept of species must be arbitrary and ruled by practical considerations, the species including a large group of distinct strains. Any one strain is capable of precise definition within certain limits, established by the degree of refinement of environmental control and biometrical accuracy, but within these limits it is indistinguishable from other strains which theoretically may exist, but cannot be recognized.

Our cultures were all grown upon Hevea-leaf agar with native brown sugar, the latter being the boiled-down sap of the sugar palm, *Arenga saccharifera*. Different lots of medium were fairly uniform. On the whole, the successive generations were subjected to no more environmental fluctuation than would probably obtain in the average laboratory, since temperature conditions in Asahan are very uniform. No effort was made to keep the cultures at constant temperature. The strains showed physiological differences among themselves, indicated by the different lengths of time required for the production of spores and by the varying abundance of spores produced, but the differences were not correlated with morphological characters. At the outset of the work it appeared that the ratio of length to breadth might be a more useful character in the differentiation of the strains than mere length, but the breadth measurements could not be made with sufficient accuracy by an ordinary ocular micrometer to make them of value. In the experiments we encountered a very small number of aberrant cultures, characterized by slow growth, numerous deformed spores, and greatly shortened appendages, which we attributed at the time to careless use of mercuric chloride on the part of a native helper. Our supposition was confirmed by the immediate return to normality in the following generation of the strains affected. There was no basis for throwing these cultures out of consideration, since they could not be distinguished from extremes due to other factors. Consequently they increase the range of variation in generation means of a number of strains, and afford one reason for our statement, above, that more careful work would merely result in decreasing the difference which would be significant in proving the distinctness of strains.

In all, the comparisons involved thirty-five strains—twenty-two from rubber, seven from cocoanut, three from tea, two from oil palm, and one from betel-nut palm. Two strains were measured through only four successive generations, eight through five generations, sixteen through six, seven through seven, and two through eight generations. Since the time involved was the same for all strains, it is evident that some of them required twice as long for spore production as others. The numerical data for all cultures are summarized in tables 1 and 2.

In deciding how many demonstrably different lines were represented in our set of thirty-five isolations, we were of course concerned with making decisions as to how significant were the differences of the final means for the several strains. It is obvious from simple inspection of tables 1 and 2 that more than one strain was involved, for the generation means of each single strain fluctuated within a range of variation (on the average) of somewhat less than 3 μ in spore length, whereas the total range of variation of the generation means of the whole set of thirty-five cultures was 13.8 μ. We therefore felt justified in determining the standard deviation of generation means by throwing all the deviations together into one series, calculating the plus and minus deviations of the generation means each from its own strain mean. In this manner we determined an approximate value for the standard deviation of the generation means of an indefinitely large number of generations of any given strain as 1.23 μ for spore length and 2.13 μ for appendage length. All the strains were similar enough so that these standard deviations might be applied to any strain. In order to determine the significance of the differences between strain means, the usual formula for the standard error of the difference of means was applied:

$$\epsilon_{1.2}{}^2 = \sigma^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)$$

TABLE 1. *Statistical Data for Successive Generations of Thirty-five Strains of Pestalozzia from Five Different Hosts*

The number of measurements in each generation was 25 for each character, except in a very few instances, when either 15 or 50 measurements were made. Measurements are in μ.

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
1	Cocoanut; leaf spot	1	17–24	20.3	4–21	11.0
		2	17–23	20.2	9–21	13.8
		3	16–24	20.4	15–21	9.0
		4	16–24	19.9	3–23	10.5
		5	17–23	19.6	15–17	9.2
		6	16–21	19.0	3–21	11.6
2	Cocoanut; leaf spot	1	17–21	19.4	4–14	9.5
		2	19–24	21.0	4–20	9.5
		3	16–26	21.3	4–21	13.1
		4	17–23	19.9	6–20	12.9
		5	17–23	20.6	3–17	12.5
3	Cocoanut; leaf spot	1	17–26	22.3	17–29	22.0
		2	19–26	22.2	11–27	20.0
		3	20–24	22.3	17–31	22.2
		4	19–29	22.4	10–29	17.3
		5	19–24	21.8	7–23	15.6
		6	17–24	20.9	6–24	17.6
4	Cocoanut; leaf spot	1	20–29	23.5	17–27	20.1
		2	19–24	21.5	19–24	20.1
		3	19–26	21.9	14–26	19.7
		4	19–26	21.8	11–29	18.4
5	Cocoanut; leaf spot	1	20–29	24.4	19–32	24.4
		2	17–26	21.1	14–34	23.1
		3	17–24	22.3	13–24	20.5
		4	17–24	20.2	20–29	23.0
		5	19–26	22.2	17–27	22.1
		6	19–29	22.4	13–29	19.1
		7	17–24	20.6	11–29	19.3
		8	19–29	23.2	14–26	19.0
6	Cocoanut; leaf spot	1	19–26	22.0	17–29	22.6
		2	20–31	23.8	14–36	21.4
		3	19–30	22.9	16–29	22.8
		4	21–26	22.9	13–26	20.1
7	Cocoanut; milk from infected nut	1	20–26	22.8	14–26	18.8
		2	20–26	23.6	13–23	17.9
		3	21–27	24.2	9–21	14.6
		4	21–29	24.2	9–20	15.0
		5	21–30	25.3	14–24	18.5
		6	20–29	24.3	9–20	14.9
8	Oil palm; leaf spot	1	23–29	24.7	21–36	26.3
		2	21–30	24.7	20–43	27.2
		3	21–29	24.7	17–33	24.7
		4	21–27	24.4	19–34	26.2
		5	21–29	24.6	21–33	26.8

Table 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
9	Oil palm; leaf spot	1	24–36	30.3	16–29	22.3
		2	20–29	24.8	17–31	23.4
		3	23–33	27.7	20–33	26.4
		4	24–37	29.5	21–33	26.7
		5	23–34	29.7	9–31	19.6
10	Betel-nut palm; leaf spot	1	24–32	28.3	19–39	25.1
		2	19–29	24.5	21–31	25.0
		3	19–34	25.2	16–29	22.3
		4	24–36	30.0	21–36	28.8
		5	29–36	32.3	9–31	17.4
11	Tea; gray blight of leaf	1	20–26	23.0	19–33	25.9
		2	20–24	22.1	16–33	26.9
		3	20–29	23.3	16–31	25.2
		4	20–29	24.5	19–33	24.1
		5	20–27	23.9	21–34	26.6
		6	20–29	24.3	13–34	22.3
12	Tea; gray blight of leaf	1	21–27	24.4	21–37	26.9
		2	19–29	23.8	24–34	29.8
		3	24–36	28.4	24–40	31.0
		4	21–31	27.3	17–41	26.6
		5	21–33	27.2	21–37	29.4
13	Tea; gray blight of leaf	1	26–37	30.8	24–43	35.8
		2	23–34	28.5	20–42	31.7
		3	23–36	27.4	21–37	29.0
		4	24–39	28.6	21–39	30.7
		5	27–36	30.8	21–37	29.4
		6	26–36	31.3	17–36	27.9
14	Hevea; wood of sapling above wound	1	17–23	19.8	21–36	26.5
		2	16–20	18.5	16–34	24.7
		3	21–27	23.0	14–36	22.5
		4	17–26	20.7	21–37	30.0
		5	19–26	21.8	16–36	25.3
		6	16–24	21.0	17–36	26.1
		7	17–23	20.4	16–29	21.3
15	Hevea; leaves white and dry at ends and borders	1	20–27	23.2	20–32	25.2
		2	19–23	20.9	14–31	21.9
		3	17–27	21.0	21–33	25.6
		4	19–24	21.2	16–30	22.9
		5	19–23	20.8	14–31	22.3
		6	20–27	23.0	14–33	20.4
16	Hevea; healthy young leaves	1	17–24	20.4	13–32	21.4
		2	17–24	20.4	16–29	23.2
		3	19–26	22.4	19–31	24.0
		4	21–27	23.3	16–33	22.7
		5	19–29	23.0	14–31	22.8
		6	19–27	22.9	16–30	22.6
17	Hevea; leaf spot of seedling	1	17–24	22.0	19–33	25.6
		2	17–23	21.1	17–37	23.6
		3	19–24	21.2	13–34	22.1
		4	20–29	23.4	14–36	24.1
		5	19–29	22.3	17–31	23.3
		6	17–26	22.5	14–31	23.4
		7	19–27	23.3	13–27	19.0

TABLE I (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
18	Hevea; wood of sapling above wound	1	20–26	23.4	20–32	25.6
		2	17–23	20.6	17–36	24.7
		3	20–27	22.7	14–27	20.8
		4	20–27	22.5	14–29	20.5
		5	20–26	22.5	16–31	23.2
		6	21–29	23.8	14–27	20.5
		7	19–29	22.8	13–31	21.0
19	Hevea; bark of tapping cut	1	20–27	22.3	10–26	20.2
		2	19–26	21.7	9–21	15.4
		3	19–27	22.2	16–29	21.9
		4	21–29	23.8	16–36	21.4
		5	17–29	22.8	10–26	19.4
		6	19–24	21.7	9–26	17.7
20	Hevea; wood of sapling above wound	1	19–24	21.8	19–29	23.5
		2	16–24	20.5	14–29	21.8
		3	19–24	20.9	14–27	22.1
		4	19–26	22.7	14–27	20.5
		5	21–33	25.3	16–30	22.6
		6	19–29	23.6	6–26	14.0
21	Hevea; colorless area of young leaf	1	19–27	23.0	11–29	20.5
		2	16–24	19.9	4–20	10.0
		3	20–26	22.4	20–34	25.0
		4	19–29	23.2	14–30	20.3
		5	21–29	24.3	11–30	20.5
		6	21–29	23.9	14–30	20.8
22	Hevea; untapped bark of large tree	1	21–32	25.4	14–27	20.5
		2	20–24	21.7	16–27	20.1
		3	20–26	23.0	13–26	19.0
		4	17–26	22.5	14–31	21.8
		5	23–29	24.8	14–27	21.1
		6	17–26	22.5	14–29	20.4
		7	20–27	23.5	10–27	16.7
23	Hevea; twig of tree killed by lightning	1	19–26	23.0	17–29	23.7
		2	19–24	21.9	17–31	21.3
		3	19–27	22.5	13–27	21.0
		4	21–30	24.9	13–30	18.5
		5	20–27	23.3	10–23	16.7
24	Hevea; untapped bark of large tree	1	20–26	23.4	13–26	19.6
		2	20–24	22.9	10–21	17.5
		3	17–27	22.9	14–24	20.4
		4	20–29	23.2	14–29	19.9
		5	20–29	22.8	14–29	18.2
		6	21–29	24.9	10–21	17.7
		7	20–26	23.5	11–24	18.2
25	Hevea; twig	1	20–27	23.0	17–29	21.6
		2	21–26	23.3	14–27	22.0
		3	17–26	21.8	19–30	25.0
		4	20–29	23.4	19–31	24.9
		5	21–27	24.6	14–31	22.9
26	Hevea; wood of sapling above wound	1	19–26	21.1	19–33	23.9
		2	17–23	20.1	14–30	21.6
		3	20–27	23.0	16–33	23.9
		4	21–29	24.4	16–30	23.7
		5	20–29	24.7	14–39	23.6
		6	23–29	26.6	14–31	20.1

TABLE 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
27	Hevea; falling young leaves infected with leaf-fall fungus..	1	20–26	21.8	20–34	27.9
		2	17–29	22.9	14–36	21.0
		3	20–29	24.5	16–30	24.8
		4	21–27	24.3	16–33	21.9
		5	21–30	24.7	16–34	22.4
		6	21–29	23.7	14–29	20.6
		7	21–29	25.5	13–21	17.9
28	Hevea; bark of large branch, killed by lightning..........	1	20–26	23.0	24–32	27.0
		2	19–26	22.2	20–57	31.4
		3	21–27	24.0	19–30	23.3
		4	21–29	25.2	14–36	22.6
		5	21–29	25.6	9–29	18.3
		6	20–30	24.7	13–34	23.1
29	Hevea; wood ten inches above old wound.................	1	21–29	23.6	20–30	24.5
		2	16–27	21.7	10–27	21.7
		3	21–30	25.6	16–30	23.1
		4	17–26	22.2	20–36	26.7
		5	21–29	25.1	20–39	25.1
		6	23–30	25.9	14–39	22.4
		7	21–30	25.5	16–33	24.0
		8	21–29	24.8	19–31	24.1
30	Hevea; falling young leaves infected with leaf-fall fungus..	1	20–29	23.9	17–33	26.1
		2	20–29	23.6	19–36	25.9
		3	20–29	24.4	16–36	24.0
		4	23–33	25.1	17–43	25.8
		5	20–30	24.7	14–31	23.0
		6	21–31	25.0	14–43	22.9
31	Hevea; old, yellow leaves.....	1	23–29	26.3	16–32	20.2
		2	21–29	25.6	14–33	21.6
		3	21–29	25.2	17–41	23.8
		4	21–31	26.0	19–30	23.3
		5	21–29	24.2	19–36	24.4
		6	19–30	24.1	17–29	22.4
32	Hevea; young leaves of cuttings with leaf-fall disease........	1	20–27	24.5	21–29	23.7
		2	21–30	26.1	16–27	21.5
		3	21–29	25.2	17–30	22.5
		4	23–29	25.5	16–33	22.5
		5	23–30	26.3	17–27	22.7
		6	23–29	26.4	16–30	23.1
33	Hevea; healthy young leaves..	1	24–29	26.0	10–17	12.7
		2	21–29	25.9	7–17	12.2
		3	23–31	27.1	4–17	11.9
		4	21–30	25.5	8–21	13.3
		5	24–31	27.5	9–23	15.1
		6	23–30	25.6	9–19	14.3
		7	23–37	27.3	11–20	15.8

TABLE 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
34	Hevea; leaf spot of mature leaves....................	1	23–32	27.5	20–29	25.2
		2	21–30	25.2	14–34	23.8
		3	21–36	28.4	21–36	27.5
		4	23–30	26.6	16–31	23.7
		5	21–31	27.4	16–31	22.2
		6	23–31	28.6	13–23	17.5
35	Hevea; leaf spot of mature leaves.....................	1	26–36	29.0	16–29	22.9
		2	24–37	29.0	16–36	26.7
		3	21–29	25.4	14–31	21.1
		4	20–29	26.1	16–34	25.7
		5	24–31	28.5	20–33	27.3

TABLE 2. *Summary of Statistical Data (All Measurements and Standard Deviations in μ)*
Part 1. Spore Length

Strain	No. of Generations	Total Range of Variation	Range of Generation Means	Mean	Standard Deviation	Coefficient of Variation
1......	6	8.0	1.4	19.9	1.88	9.5
2......	5	10.0	1.9	20.6	1.85	9.0
3......	6	12.0	1.5	22.0	1.89	8.6
4......	4	10.0	2.0	22.0	1.92	8.7
5......	8	12.0	4.2	22.0	2.11	9.6
6......	4	12.0	1.8	22.9	2.33	10.2
7......	6	10.0	2.5	24.2	1.98	8.2
8......	5	9.0	0.3	24.6	2.03	8.3
9......	5	17.0	5.5	28.3	3.60	10.3
10......	5	17.0	7.8	28.0	3.90	13.9
11......	6	9.0	2.4	23.4	2.23	9.5
12......	5	17.0	4.6	26.4	3.10	11.7
13......	6	16.0	3.9	29.4	3.31	11.3
14......	7	11.0	4.5	20.7	2.16	10.4
15......	6	10.0	2.4	21.6	2.01	9.3
16......	6	12.0	2.9	22.0	2.34	10.6
17......	7	12.0	2.3	22.3	2.17	9.8
18......	7	12.0	3.2	22.4	2.00	8.9
19......	6	12.0	2.1	22.5	2.28	10.1
20......	6	17.0	4.8	22.5	2.82	12.5
21......	6	13.0	4.4	23.0	2.51	11.0
22......	7	15.0	3.7	23.0	2.17	9.4
23......	5	10.0	3.0	23.2	2.34	10.1
24......	7	12.0	2.1	23.3	2.00	8.6
25......	5	12.0	2.8	23.3	2.02	8.7
26......	6	12.0	6.5	23.5	2.94	12.5
27......	7	13.0	3.7	23.9	2.35	9.8
28......	6	11.0	3.4	24.2	2.34	9.7
29......	8	14.0	4.2	24.3	2.67	11.0
30......	6	13.0	1.5	24.5	2.28	9.3
31......	6	12.0	2.2	25.2	2.27	9.0
32......	6	10.0	1.9	25.9	2.11	8.1
33......	7	16.0	2.0	26.3	2.38	9.1
34......	6	15.0	3.4	27.3	2.56	9.4
35......	5	16.0	3.6	27.3	2.82	10.3

Part 2. Appendage length

Strain	No. of Generations	Total Range of Variation	Range of Generation Means	Mean	Standard Deviation	Coefficient of Variation
1......	6	22.0	4.8	10.9	4.54	41.6
2......	5	19.0	3.6	11.6	4.60	39.7
3......	6	26.0	6.6	18.9	4.42	23.3
4......	4	18.0	1.7	19.7	3.42	17.4
5......	8	23.0	5.4	21.3	3.96	18.1
6......	4	23.0	2.7	21.6	3.69	17.1
7......	6	17.0	4.2	16.4	3.47	21.2
8......	5	26.0	2.5	26.3	4.45	16.8
9......	5	24.0	7.1	23.7	4.56	19.2
10......	5	30.0	11.4	23.6	5.86	24.8
11......	6	21.0	4.6	25.2	4.78	19.0
12......	5	24.0	4.4	28.7	4.15	14.5
13......	6	26.0	7.9	30.0	4.73	15.8
14......	7	23.0	8.7	25.1	5.05	20.1
15......	6	19.0	5.2	23.0	4.03	17.5
16......	6	20.0	2.6	22.8	3.93	17.2
17......	7	24.0	6.6	22.9	5.33	23.3
18......	7	23.0	5.1	22.0	4.13	18.8
19......	6	27.0	6.5	19.4	4.22	21.8
20......	6	24.0	9.5	20.6	4.13	20.1
21......	6	30.0	10.8	19.4	6.38	32.9
22......	7	21.0	5.1	19.9	3.95	19.9
23......	5	21.0	7.0	19.9	4.60	23.1
24......	7	19.0	2.7	18.7	3.45	18.5
25......	5	16.0	3.4	23.5	4.09	17.0
26......	6	25.0	3.8	22.7	4.36	19.2
27......	7	23.0	10.0	21.5	4.44	20.7
28......	6	48.0	13.1	24.2	5.05	20.9
29......	8	29.0	5.0	24.0	3.96	16.5
30......	6	29.0	3.2	24.6	4.96	21.6
31......	6	27.0	2.8	22.9	4.25	18.6
32......	6	17.0	2.2	22.6	3.36	14.9
33......	7	19.0	3.9	13.4	3.10	23.1
34......	6	23.0	10.0	23.3	5.01	21.5
35......	5	22.0	6.2	24.6	4.93	20.0

The least number of generations upon which any strain mean was based was four, the greatest, eight. The least accurate comparisons, therefore, would be between two strains both of which had been carried only four generations. Conversely, the most accurate comparison would be between strains which had both been carried eight generations. The standard errors of the differences between the means of the several strains were multiplied by three, and if an observed difference between two strains exceeded this value, the difference in question was considered to demonstrate the probable distinctness of the two strains. For spore length 1.8 μ was considered a significant deviation in the case of the most dependable comparisons; 2.7 μ in the case of the least dependable. The corresponding criteria for the appendage lengths were 3.1 μ and 4.5 μ. It must be remembered that a criterion of three times the standard error of a difference between means secures practical certainty of the distinctness of strains. On the contrary, it still remains highly probable that unlike strains remain in association in the unresolved groups, which might be resolved further by more careful work.

Turning now to an analysis of the data presented in the tables, it appears that there are two strains, nos. 13 and 33, each of which cannot be like any other strain in the series. Two others, nos. 1 and 2, fall together and cannot be thrown with any other group. No. 12 can be associated only with no. 35. However, nos. 9, 10, and 34 form a natural group, with which no. 35 can also be associated. The latter, falling between no. 12 and the group composed of nos. 9, 10, and 34, can be placed with either, and it is of course a matter of judgment where it should go. No. 12 has the appendages consistently longer than the spores, whereas in nos. 9, 10, 34, and 35 the appendages are consistently shorter than the spores. A group consisting of nos. 9, 10, 34, and 35 is therefore adopted, leaving no. 12 to stand alone.

Applying our criteria to nos. 34 and 35, we find that they might have been associated with nos. 31 and 32 if they had not been placed with nos. 9 and 10. We have therefore left nos. 31 and 32 to be associated with other strains, which prove to be nos. 8, 28, 29, and 30. We might therefore segregate a group consisting of these six strains. Comparing the generation means for spore length and appendage length, however, we find that there is decided justification for considering that such a group would constitute an unnatural assemblage. In nos. 31 and 32 the spores are consistently, generation after generation, longer than the appendages; in no. 8 the exact contrary is the case; in nos. 28, 29, and 30 the spores and appendages are of the same mean length. Such consistent correlations cannot be looked upon as due to mere chance, and we are therefore forced to consider nos. 31 and 32 as constituting one group, and no. 8 another. Nos. 28, 29, and 30 come next to no. 7 in spore length, and this form proves distinct on the basis of appendage length. The next shorter strain, no. 27, proves to be distinct from no. 32 as to spore length. We have no choice, then, other than to consider nos. 28, 29, and 30 as a group.

The remaining twenty strains offer more difficult problems. Probably no two persons would group them in exactly the same way, but the results would be very similar. Tabulating the possible likenesses brings out the fact that there are three outstanding strains which are difficult to associate with any considerable number of others. These are nos. 7, 11, and 14. No. 7 falls just on the border of an otherwise relatively uniform group consisting of nos. 3, 4, 19, 21, 22, 23, and 24, and may be thrown with them or kept apart, as seems preferable. We consider it a distinct strain. Nos. 26 and 27 fall together, and cannot be combined with the large group last mentioned because of the significant difference between nos. 26 and 24. Nos. 11 and 25 are not particularly alike, but the data do not show a significant difference between them. Nos. 5, 6, 15, 16, 17, 18, and 20 form a relatively uniform group. No. 14 is best kept by itself. If it is thrown into one of the other groups it necessitates the removal of some other strain or strains from that group, and on the whole no. 14 seems the most outstanding one to be left outside the group.

This rough analysis shows us that our 35 strains fall into the following fourteen groups:

I. Strains 1 and 2, from leaf spot of cocoanut palm. Spores about 20.2 μ; appendages 11.2 μ.
II. Strains 3 and 4, from leaf spot of cocoanut palm; nos. 19, 22, and 24, from bark of trunk of Hevea; nos. 21 and 23, from leaf and twig of Hevea, respectively. Spores about 22.7 μ; appendages 19.4 μ.
III. Strains 5 and 6, from leaf spot of cocoanut palm; nos. 15, 16, and 17, from Hevea leaves; nos. 18 and 20, from wood of Hevea sapling above wound. Spores about 22.2 μ; appendages 22.0 μ.
IV. Strain 7, from milk of infected cocoanut, drawn from fruit under sterile conditions. Spores about 24.2 μ; appendages 16.4 μ.
V. Strain 8, from leaf spot of oil palm. Spores about 24.6 μ; appendages 26.3 μ.
VI. Strain 9, from leaf spot of cocoanut palm; no. 10, from leaf spot of betel-nut palm; nos. 34 and 35, from leaf spot of Hevea leaves. Spores about 27.7 μ; appendages 23.8 μ.
VII. Strains no. 11, from gray blight of tea (leaf), and no. 25, from Hevea twig. Spores about 23.3 μ; appendages 24.3 μ.
VIII. Strain 12, from gray blight of tea (leaf). Spores about 26.4 μ; appendages 28.7 μ.
IX. Strain 13, from gray blight of tea (leaf). Spores about 29.4 μ; appendages 30.0 μ.
X. Strain no. 14, from wood of Hevea sapling above wound. Spores about 20.7 μ; appendages 25.1 μ.
XI. Strains 26 and 27, from wood of sapling Hevea and young leaf of Hevea, respectively. Spores about 23.7 μ; appendages 22.1 μ.
XII. Strains 28, 29, and 30, from bark of large Hevea branch, killed by lightning, wood of Hevea sapling, and young Hevea leaf, respectively. Spores about 24.3 μ; appendages 24.3 μ.
XIII. Strains 31 and 32, from Hevea leaves. Spores about 25.5 μ; appendages 22.8 μ.
XIV. Strain 33, from young Hevea leaf. Spores about 26.2 μ; appendages 13.4 μ.

SUMMARY

1. The tropical Pestalozzias of such hosts as the cocoanut palm, Hevea, tea, etc., have not been critically differentiated by past authors, the names *P. palmarum* and *P. Guepini* being used according to individual preference for one or the other. These supposed species are incapable of definition.

2. Statistical studies of thirty-five isolations from cocoanut palm, oil palm, betel-nut palm, Hevea, and tea, show that numerous strains, morphologically distinct from one another, may be isolated, and that these strains do not appear to be confined to particular hosts.

3. It appears that by using a sufficiently refined technique a nominal species such as *Pestalozzia Guepini* might be resolved into an indefinite number of demonstrably distinct strains, the number depending only upon the precision of the methods. In illustration, we have shown a possible allocation of our thirty-five strains to fourteen groups, each of which contains one or more strains that cannot be placed in any other group.

4. The species concept in the Fungi Imperfecti is of necessity a highly artificial one. The ultimate unit, an impracticable one for purposes of classification, is the pure line descended from a single spore.

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